AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

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Optimal Periprosthetic Tissue Specimen Number for Diagnosis of Prosthetic Joint Infection

Trisha N. Peel,[a,b] Tim Spelman,[c] Brenda L. Dylla,[a] John G. Hughes,[a] Kerryl E. Greenwood-Quaintance,[a] Allen C. Cheng,[b,d] Jayawant N. Mandrekar,[e] Robin Patel[a,f]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA[a]; Department of Infectious Diseases, Monash University and Alfred Hospital, Melbourne, Australia[b]; Department of Surgery, St. Vincent's Hospital Melbourne, University of Melbourne, Melbourne, Australia[c]; Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia[d]; Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA[e]; Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA[f]

ABSTRACT We recently demonstrated improved sensitivity of prosthetic joint infection (PJI) diagnosis using an automated blood culture bottle system for periprosthetic tissue culture [T. N. Peel et al., mBio 7(1):e01776-15, 2016, https://doi.org/10.1128/mBio.01776-15]. This study builds on the prior research by examining the optimal number of periprosthetic tissue specimens required for accurate PJI diagnosis. Current guidelines recommend five to six, which is impractical. We applied Bayesian latent class modeling techniques for estimating diagnostic test properties of conventional culture techniques (aerobic and anaerobic agars and thioglycolate broth) compared to inoculation into blood culture bottles. Conventional, frequentist receiver operating characteristic curve analysis was conducted as a sensitivity analysis. The study was conducted at Mayo Clinic, Rochester, MN, from August 2013 through April 2014 and included 499 consecutive patients undergoing revision arthroplasty from whom 1,437 periprosthetic tissue samples were collected and processed. For conventional periprosthetic tissue culture techniques, the greatest accuracy was observed when four specimens were obtained (91%; 95% credible interval, 77 to 100%), whereas when using inoculation of periprosthetic tissues into blood culture bottles, the greatest accuracy of diagnosis was observed when three specimens were cultured (92%; 95% credible intervals, 79 to 100%). Results of this study show that the greatest accuracy of PJI diagnosis is obtained when three periprosthetic tissue specimens are obtained and inoculated into blood culture bottles or four periprosthetic tissue specimens are obtained and cultured using standard plate and broth cultures. Increasing the number of specimens to five or more, per current recommendations, does not improve accuracy of PJI diagnosis.

KEYWORDS blood culture bottles, periprosthetic tissue, prosthetic joint infection

An estimated 1.3 million prosthetic hip or knee replacement procedures were performed in 2015 in the United States (1). Failure of the prosthesis, necessitating revision surgery, occurs in approximately 10% of patients, with an estimated 140,000 prosthetic hip or knee revision procedures performed in 2015 in the United States (1). The major indication for revision of the prosthesis is mechanical or aseptic failure; however, in 1 to 3% of patients undergoing prosthetic joint replacement surgery, the prosthesis is revised for "septic failure" or prosthetic joint infection (PJI) (2). Despite recent advances in diagnosis, differentiation of aseptic failure from septic failure, which is critical to guide antimicrobial therapy, may in some cases be challenging (2).

Received 15 September 2016 **Returned for modification** 10 October 2016 **Accepted** 1 November 2016

Accepted manuscript posted online 2 November 2016

Citation Peel TN, Spelman T, Dylla BL, Hughes JG, Greenwood-Quaintance KE, Cheng AC, Mandrekar JN, Patel R. 2017. Optimal periprosthetic tissue specimen number for diagnosis of prosthetic joint infection. J Clin Microbiol 55:234–243. https://doi.org/10.1128/JCM.01914-16.

Editor B. A. Forbes, Virginia Commonwealth University Medical Center

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Address correspondence to Robin Patel, patel.robin@mayo.edu.

Microbiological culture of the periprosthetic tissue is the mainstay for PJI diagnosis (2, 3). Indeed, microbiological findings are included as a major criterion in both the Musculoskeletal Infection Society (MSIS) and the Infectious Diseases Society of America (IDSA) diagnostic criteria for PJI (3, 4).

The optimal number of specimens required to enable accurate diagnosis of PJI has been examined in a number of studies; the most highly cited is the study by Atkins and colleagues, published in 1998 (5). Applying histopathological criteria as the reference standard, Atkins et al. examined the optimal number of specimens required to diagnose PJI. Utilizing mathematical modeling, the authors noted that the number of specimens needed to recover the same organism from three or more specimens for optimal sensitivity exceeded seven, which the authors concluded was impractical (5). Therefore, they recommended that five to six specimens be obtained, with a cutoff of two or more specimens yielding the same organism used to diagnose PJI (5). The isolation of an indistinguishable microorganism from two or more periprosthetic specimens is incorporated in the MSIS and IDSA criteria, and it is recommended that ideally five or six periprosthetic samples be obtained at revision surgery (3, 4).

There are limited data on the optimal culture media for periprosthetic tissue culture, and practices are not standardized across clinical laboratories (2, 5, 6). In the study by Atkins et al., periprosthetic tissue samples were cultured on chocolate and blood agars and in Robertson's cooked meat broth (5).

Inoculation of periprosthetic samples into blood culture bottles has recently been described as being potentially more sensitive than culture performed using conventional techniques (6, 7). In light of this, we undertook a large, prospective cohort study comparing the performance of aerobic and anaerobic agars, thioglycolate broth, and aerobic and anaerobic blood culture bottles for periprosthetic tissue culture (8). Inoculation of tissues into blood culture bottles was associated with increased sensitivity compared to conventional agar and broth cultures applying Bayesian latent class modeling (92% [95% credible interval, 85 to 97%] versus 63% [95% credible interval, 52 to 73%], respectively) and using the more traditional frequentist analysis (61% [95% confidence interval, 51 to 70%] versus 44% [95% confidence interval, 35 to 53%], respectively; $P = 0.003$) (8). The specificity of culture in blood culture bottles was similar to that of conventional media (8). Together, these studies support the inoculation of periprosthetic tissue specimens into blood culture bottles to improve the diagnostic yield of microbiological culture (6–8). Whether this improved performance influences the requisite number of tissues to be cultured is, however, unknown.

The aim of this current study was to reevaluate the optimal number of periprosthetic specimens for the diagnosis of PJI, comparing conventional culture techniques to the newer technique of inoculation of specimens into blood culture bottles using the data from our recently published study (8).

RESULTS

Over the study period, 499 patients underwent revision arthroplasty and had microbiological samples submitted for culture. Applying the MSIS definition for PJI, 111 (22%) met the definition for PJI; when microbiological findings were omitted from these criteria, 75 (15%) still met the modified definition for PJI. Demographic characteristics for the cohort are outlined in Table 1 . A majority of the subjects underwent revision surgery on hip or knee prostheses (83%). Overall, 1,837 tissue or fluid specimens were collected and processed. Of these, 1,437 deep prosthetic tissue specimens were obtained and 1,419 pairs of blood culture bottles were inoculated. Subjects had a median of three (interquartile range [IQR], 2 to 3) periprosthetic tissue specimens with a median of three blood culture bottle pairs inoculated per patient (IQR, 2 to 3). In total, subjects had a median of four microbiological specimens obtained (IQR, 3 to 4), including one or more synovial fluid specimens in 223 patients (45%) and sonicate fluid culture in 102 subjects (20%). There was an observed difference in total number of specimens collected between patients meeting the MSIS definition of PJI and those who did not (Table 2); patients meeting the criteria for PJI had a median of five specimens (IQR, 4

TABLE 1 Demographic, perioperative biochemical, microbiological, and histopathological characteristics of study subjects

Variable	MSIS criteria for PJI present		*P* value
	No ($n = 388$)	Yes ($n = 111$)	
Median age, yr (IQR)	63 (56–73.5)	63 (57–71)	0.991
Female sex, no. (%)	206 (53)	43 (39)	0.008
Prosthetic joint type, no. (%)			
Hip	160 (41)	31 (28)	
Knee	173 (45)	51 (46)	<0.001
Shoulder	44 (11)	16 (14)	
Elbow	11 (3)	13 (12)	
Prior revision surgery on index joint, no. (%)	162 (43)	71 (65)	<0.001
Median no. of prior revision surgeries on index joint (IQR)	0 (0–1)	1 (0–2)	<0.001
Documented history of PJI, no. (%)	111 (29)	62 (56)	<0.001
Median no. of days of symptoms prior to surgery (IQR)	271.5 (124–594)	113.5 (39–345)	<0.001
Median implant age, days (IQR)	1,181 (437–3,219)	463 (186–1,306)	<0.001
Presenting symptoms, no. (%)			
Pain	311 (80)	88 (79)	0.839
Erythema along incision	5 (1)	23 (21)	<0.001
Swelling	24 (6)	32 (29)	<0.001
Drainage from the incision	1 (0.3)	43 (39)	<0.001
Sinus tract	0 (0)	37 (33)	
Fever	6 (2)	26 (14)	<0.001
Antibiotics in 4 wk prior to surgery, no. (%)	50 (13)	57 (51)	<0.001
Surgery performed, no. (%)			
Revision arthroplasty (including one-stage exchange)	264 (68)	11 (10)	
Resection arthroplasty (with or without arthrodesis)	0 (0)	1 (1)	
Resection with insertion of a spacer as part of two-stage exchange	34 (9)	66 (59)	<0.001
Debridement and implant retention	14 (4)	27 (25)	
Reimplantation as part of two-stage exchange	76 (20)	4 (4)	
Amputation	0 (0)	2 (2)	
Median level of pre-operative C-reactive protein, mg/liter (IQR)	3.1 (3–6.5)	25.3 (9.3–55.9)	<0.001
Median preoperative erythrocyte sedimentation rate, mm/h (IQR)	9 (4–18)	30.5 (17.5–56)	<0.001
Preoperative synovial fluid aspiration performed, no. (%)	116 (30)	60 (54)	<0.001
Median synovial fluid leukocytes, no./μl (IQR)	872 (355–1,920)	35,792 (13,453–82,341)	<0.001
Median synovial fluid neutrophil, % (IQR)	13 (5–51)	91 (85–96)	<0.001
Positive synovial fluid culture, no. (%)	7 (7)	45 (73)	<0.001
Pre- and/or intraoperative synovial fluid culture performed, no. (%)	162 (42)	61 (55)	0.014
Positive synovial fluid culture, no. (%)	9 (6)	42 (69)	<0.001
Sonicate culture performed, no. (%)	68 (18)	34 (31)	0.003
Positive sonicate culture, no. (%)	1 (1)	23 (68)	<0.001
Histopathology specimen obtained, no. (%)	307 (79)	65 (59)	<0.001
Median no. of periprosthetic specimens obtained (IQR)	3 (3–4)	5 (4–6)	<0.001
Median no. of periprosthetic tissue cultures performed (IQR)	3 (2–3)	3 (3–4)	<0.001

to 6), compared to a median of three specimens (IQR, 3 to 4) in patients not meeting the definition (rank sum test, $P < 0.0001$).

A microorganism was isolated in two or more specimens in 89 of the 111 PJI cases (80%) (Table 3). Staphylococci were the most common organisms isolated in two or more specimens, being found in 48% of PJI cases. In 10 PJI cases, a microorganism was isolated in a single specimen, including two PJI cases that yielded *Staphylococcus aureus* from a single specimen. Cultures were negative in 12 PJI cases (11%).

Using conventional methods for culture of periprosthetic tissues, synovial fluid, and sonicate fluid, a microorganism(s) was isolated from two or more specimens in 72 (65%) of PJI cases. A single culture was positive in 55 patients (14%) of patients not meeting the definition of PJI (false positive).

TABLE 2 Number and percentage of specimens (periprosthetic tissue, synovial fluid, and/or sonicate fluid specimens) submitted for microbiological culture per subject according to the MSIS criteria for PJI

No. of specimens/ subject	No. (%) of specimens		
	No PJI (n = 388)	PJI (n = 111)	Total (n = 499)
1	36 (9.3)	0 (0)	36 (7.2)
2	30 (7.7)	4 (3.6)	34 (6.8)
3	162 (41.8)	16 (14.4)	178 (35.7)
4	101 (26.0)	32 (28.8)	133 (26.7)
5	42 (10.8)	29 (26.1)	71 (14.2)
6	10 (2.6)	17 (15.3)	27 (5.4)
7	3 (0.8)	9 (8.1)	12 (2.4)
8	2 (0.5)	4 (3.6)	6 (1.2)
9	2 (0.5)	0 (0)	2 (0.4)

In addition to synovial and sonicate fluid cultures, when periprosthetic tissue specimens were inoculated into blood culture bottles, a microorganism(s) was isolated from two or more specimens in 79 (71%) of 111 PJI cases. A single culture was positive in 58 of 388 (15%) subjects not meeting the definition of PJI.

Looking specifically at culture of periprosthetic tissue specimens, using conventional culture methods, a microorganism(s) was isolated from two or more specimens in 55 (50%) PJI cases (Table 4), and single specimens were culture positive in 44 (11%) subjects not meeting the definition of PJI. Using culture of periprosthetic tissues in blood culture bottles, a microorganism(s) was isolated from two or more specimens in 72 (65%) PJI cases, whereas in patients not meeting the definition of PJI, a single specimen inoculated in blood culture bottles was positive in 49 (13%) subjects.

The maximum number of PJI cases detected was observed when up to eight specimens (including periprosthetic tissue, synovial fluid, and the implant) were submitted and processed using conventional techniques and culture of periprosthetic tissue in blood culture bottles. In examining periprosthetic tissue specimens alone, the greatest number of infected cases was detected when up to six specimens were processed with conventional culture methods. When adopting the new culture technique of inoculation of periprosthetic tissues into blood culture bottles, the maximum number of PJI cases was detected when up to six specimens were inoculated; increasing the number of specimens beyond six did not improve the diagnostic yield but did result in increasing numbers of false-positive results.

The sensitivity, specificity, and accuracy of increasing microbiological specimen numbers according to the different culture media and methods are outlined in Table 5 . For the conventional culture technique, including synovial and sonicate fluid culture (when performed), the greatest sensitivity, adopting latent class analysis modeling, was observed when up to six specimens were cultured (98%; 95% credible interval, 93 to 100%); however, this was associated with low specificity (45%; 95% credible interval, 25 to 68%). The greatest estimated accuracy was observed when up to five specimens were obtained (using Bayesian latent class modeling) compared to up to seven specimens (when frequentist analysis was performed).

For periprosthetic tissue culture in blood culture bottles, including synovial and sonicate fluid culture (when performed), the greatest sensitivity, adopting latent class analysis modeling, was observed when up to five specimens were cultured (98%; 95% credible interval, 94 to 100%); however, the specificity was very poor (10%; 95% credible interval, 1 to 27%). This was mirrored for the frequentist analysis. The greatest estimated accuracy was observed when up to three specimens were obtained (using Bayesian latent class modeling) (accuracy, 93%; 95% credible interval, 80 to 100%).

When examining only the number of periprosthetic tissue specimens, for the conventional periprosthetic tissue culture techniques, the greatest sensitivity, adopting latent class analysis modeling, was observed when up to five specimens were cultured

TABLE 3 Microbiological culture results for patients meeting the MSIS definition of PJI[a]

Microorganism(s) or result type	No. (%) (n = 111)
Staphylococcus species	47 (42)
Staphylococcus aureus	22 (22)
Staphylococcus epidermidis	20 (18)
Staphylococcus lugdunensis	2 (2)
Staphylococcus capitis	1 (1)
Staphylococcus hominis	1 (1)
Staphylococcus saccharolyticus	1 (1)
Streptococcus species	4 (4)
Streptococcus agalactiae	1 (1)
Streptococcus bovis group	1 (1)
Streptococcus gordonii	1 (1)
Streptococcus group G	1 (1)
Enterococcus faecalis	2 (2)
Other Gram-positive cocci	
Granulicatella adiacens	2 (2)
Facklamia hominis	1 (1)
Finegoldia magna	1 (1)
Parvimonas micra	2 (2)
Gram-positive bacilli	14 (13)
Corynebacterium amycolatum	1 (1)
Propionibacterium acnes	12 (11)
Propionibacterium granulosum	1 (1)
Gram-negative bacilli	8 (7)
Escherichia coli	1 (1)
Proteus mirabilis	1 (1)
Enterobacter cloacae	1 (1)
Pseudomonas aeruginosa	3 (3)
Serratia marcescens	1 (1)
Stenotrophomonas maltophilia	1 (1)
Candida albicans	1 (1)
Polymicrobial	7 (6)
S. aureus, *P. acnes*	1 (1)
S. aureus, *Porphyromonas bennonis*	1 (1)
S. aureus, *S. agalactiae*, *Enterobacter aerogenes*, *Actinomyces* species, *Peptoniphilus* species, *Anaerococcus vaginalis*, *Trueperella bernardiae*	1 (1)
S. epidermidis, *E. faecalis*	1 (1)
S. epidermidis, *P. acnes*	2 (2)
Streptococcus sanguis, *Haemophilus parainfluenzae*, *Veillonella* species	1 (1)
Indeterminate (microorganism isolated in single specimen only)	10 (9)
S. aureus	2 (2)
Staphylococcus warneri	1 (1)
Staphylococcus caprae	1 (1)
S. epidermidis, *Micrococcus luteus*	1 (1)
Corynebacterium jeikeium	2 (2)
Corynebacterium striatum	1 (1)
C. amycolatum, *Cellulosimicrobium cellulans*	1 (1)
S. bovis group, *P. acnes*	1 (1)
Culture negative	12 (11)

[a]Based on the microbiological culture results of all culture methods and media.

(98%; 95% credible interval, 91 to 100%); however, this was associated with the lowest specificity (22%; 95% credible interval, 7 to 46%). Similar results were obtained when applying frequentist analysis. The greatest estimated accuracy was observed when up to four specimens were obtained (using Bayesian latent class modeling) compared to up to three specimens (when frequentist analysis was performed).

For culture of periprosthetic tissues in blood culture bottles, the greatest sensitivity,

TABLE 4 Number of subjects with positive culture results with increasing number of specimens collected according to the different culture media and methods[a]

No. of specimens	No. of positive cultures/total no. of specimens by method											
	Conventional periprosthetic tissue culture and synovial fluid and sonicate fluid culture			Culture of periprosthetic tissue in blood culture bottles and synovial fluid and sonicate fluid culture			Conventional periprosthetic tissue culture			Periprosthetic tissue culture in blood culture bottles		
	PJI (n = 111)		No PJI (n = 388); 1 specimen culture positive	PJI (n = 111)		No PJI (n = 388); 1 specimen culture positive	PJI (n = 111)		No PJI (n = 388); 1 specimen culture positive	PJI (n = 111)		No PJI (n = 388); 1 specimen culture positive
	≥1 specimen culture positive	≥2 specimens culture positive		≥1 specimen culture positive	≥2 specimens culture positive		≥1 specimen culture positive	≥2 specimens culture positive		≥1 specimen culture positive	≥2 specimens culture positive	
1	0/0	0/0	1/36	0/0	0/0	4/23	0/0	0/0	1/35	1/1	0/0	5/35
≤2	4/4	4/4	4/66	3/4	3/4	7/51	13/15	8/15	7/112	15/20	11/19	10/112
≤3	18/20	13/20	21/228	16/20	14/20	26/212	51/66	35/66	32/323	60/73	51/72	39/324
≤4	47/52	34/52	37/329	43/52	37/51	42/313	62/84	42/84	39/354	70/89	59/88	47/355
≤5	71/81	52/81	49/371	69/81	59/80	53/355	79/106	52/106	42/368	85/108	71/107	49/369
≤6	85/98	63/98	51/381	83/98	72/97	56/365	82/110	55/110	42/369	86/110	72/109	49/321
≤7	92/107	70/107	52/384	90/107	78/106	57/368	82/110	55/110	42/370	86/110	72/109	49/370
≤8	94/111	72/111	53/386	93/111	79/110	57/313	82/111	55/111	44/372	87/111	72/110	49/372
≤9	94/111	72/111	55/388	93/111	79/110[b]	58/372[c]	82/111	55/111	44/372	87/111	72/110[b]	49/372[c]

[a]Results are reported as the number of cultures positive/total number of specimens for each increasing stratum in patients with and without PJI.
[b]One patient with PJI had only a single specimen inoculated into the blood culture bottles.
[c]Sixteen patients without PJI did not have a periprosthetic tissue specimen inoculated into the blood culture bottles.

TABLE 5 Sensitivity, specificity, and accuracy for detection of a microorganism in two or more specimens according to culture medium

Culture type and no. of specimens	Assuming no reference standard (Bayesian latent class modeling)			Modified MSIS criteria for PJI[a] (frequentist ROC analysis)		
	Sensitivity (95% credible interval)	Specificity (95% credible interval)	Accuracy (95% credible interval)	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)	Accuracy (95% confidence interval)
Conventional periprosthetic tissue culture as well as synovial and sonicate fluid culture						
≤2	—[b]	—	—	—	—	—
≤3	—	—	—	—	—	—
≤4	65 (51–79)	95 (83–100)	74 (60–85)	40 (26–56)	40 (21–61)	40 (28–52)
≤5	97 (87–100)	91 (69–100)	95 (82–100)	68 (53–81)	20 (7–41)	44 (34–56)
≤6	98 (93–100)	45 (25–68)	83 (74–91)	85 (72–94)	8 (1–26)	47 (39–54)
≤7	98 (93–100)	14 (3–31)	74 (67–80)	96 (86–100)	0 (0–14)	48 (45–51)
Periprosthetic tissue culture in blood culture bottles, as well as synovial and sonicate fluid culture						
≤2	24 (14–36)	95 (82–100)	41 (31–52)	19 (9–33)	84 (66–95)	51 (43–60)
≤3	92 (81–100)	93 (76–100)	93 (80–100)	73 (58–85)	29 (14–48)	51 (41–61)
≤4	98 (93–100)	69 (41–97)	91 (81–99)	88 (75–95)	23 (10–41)	55 (46–64)
≤5	98 (94–100)	10 (1–27)	77 (72–82)	98 (89–100)	0 (0–11)	49 (47–51)
Conventional periprosthetic tissue culture						
≤2	22 (11–37)	94 (79–100)	44 (31–56)	12 (3–28)	81 (58–95)	46 (36–57)
≤3	87 (72–99)	93 (76–100)	89 (73–100)	68 (50–83)	43 (22–66)	55 (42–69)
≤4	97 (90–100)	78 (49–99)	91 (77–100)	77 (59–89)	24 (8–47)	50 (38–62)
≤5	98 (91–100)	22 (7–46)	75 (66–84)	94 (80–99)	5 (0–24)	49 (43–56)
Periprosthetic tissue culture in blood culture bottles						
≤2	21 (12–34)	95 (82–100)	40 (29–50)	14 (5–27)	82 (63–94)	48 (39–57)
≤3	92 (80–100)	93 (76–100)	92 (79–100)	71 (55–83)	29 (13–49)	50 (39–60)
≤4	98 (93–100)	72 (44–98)	91 (81–99)	86 (73–95)	25 (11–45)	56 (46–65)
≤5	98 (94–100)	10 (1–28)	76 (70–82)	98 (88–100)	0 (0–12)	49 (47–51)

[a]Microbiological criteria omitted from the definition of PJI.
[b]—, insufficient numbers.

adopting latent class modeling analysis, was observed when up to five specimens were cultured (98%; 95% credible interval, 94 to 100%). Increasing from four to five specimens resulted in a decrease in specificity on latent class modeling (specificity, 10%; 95% credible interval, 1 to 28%). The greatest estimated accuracy was observed when up to three specimens were obtained (using Bayesian latent class modeling) (92%; 95% credible intervals, 79 to 100%).

DISCUSSION

Based on revision surgery estimates for the United States, with optimal adherence to current recommendations, a minimum of 700,000 periprosthetic specimens would have been obtained and processed at the time of revision surgery in 2015 (1). Given the projected increase in revision cases over the coming decades, this workload is set to increase significantly (1). The collection and culture of periprosthetic specimens at the time of revision surgery are important to detect or exclude the diagnosis of PJI; however, this must be considered within the broader scope of modern clinical practice. The marginal utility of improved sensitivity with the incremental increase in the number of specimens collected must be balanced against reduced specificity, in addition to resource demands placed on clinical microbiology laboratories (9).

Inoculation of periprosthetic tissue samples into blood culture bottles is associated with improved sensitivity compared to conventional strategies (6, 8). In our prior research, use of blood culture bottles for periprosthetic tissue culture was associated

with a 47% improvement in sensitivity compared to conventional culture techniques. In our study, the majority of patients had chronic infections, as evidenced by their long symptom duration prior to surgery (median, 113.5 days; interquartile range, 39 to 345 days); chronic PJI is considered more challenging to diagnose than acute PJI (2, 10). Of additional benefit, the time to microorganism detection was more rapid with culture of tissue in blood culture bottles than with conventional culture techniques (8). Adoption of the semiautomated blood culture bottle systems for PJI diagnosis has the potential additional benefit of improving laboratory workflow efficiency (9, 11).

The current study has demonstrated the greatest accuracy for PJI diagnosis when three periprosthetic tissue specimens were obtained and inoculated into blood culture bottles (applying latent class modeling). Of interest, compared to the previous study by Atkins et al. in which collection of five to six specimens was associated with the best test performance, in this current study, the greatest accuracy using conventional culture techniques for periprosthetic tissue samples was observed when four specimens were obtained, applying latent class modeling, or up to three specimens when frequentist analysis was performed (5). This difference may be a result of the different culture media selected or the statistical methodologies applied, including the "reference standard'" for the frequentist analysis (i.e., histopathological criteria compared to modified MSIS criteria).

Results of this study are similar to those of the recently published study by Bémer et al. (12) assessing the number of perioperative specimens, including periprosthetic tissue, bone, and joint fluid samples, required for the accurate diagnosis of PJI. This prospective cohort study involved 215 patients with confirmed PJI according to modified IDSA criteria (3, 12). The microbiological criterion for infection was the isolation of a "strict pathogen" in one or more cultures or the isolation of a "skin commensal" in two or more cultures (12). The authors assessed agreement between microbiological and PJI diagnostic criteria for each stratum of specimen numbers, performing 1,000 iterations of a random sample to obtain the average agreement rate. Overall, obtaining four periprosthetic specimens had the highest level of agreement, with 98.1% agreement for the microbiological criteria (95% confidence interval, 96.4 to 99.5%). As with our study, blood culture bottle inoculation of periprosthetic tissue samples was used; however, at variance, only an aerobic pediatric bottle was employed. Also at variance is that Schaedler broth was used for anaerobic culture and that patients without PJI were not included. In addition, the microbiological criteria used by Bémer et al. and in our study differed; in our study, a culture result was considered positive only if the same microorganism was isolated in two or more specimens with no reference to organism type or virulence (4, 12).

Failure of culture methods to detect an organism remains a challenge. In this study, 11% of patients meeting the revised MSIS criteria for PJI had negative cultures. Of note, patients with suspected or proven PJI were more likely than those without PJI to have received antibiotics in the 4 weeks prior to surgery (51 versus 13%, $P < 0.001$). Administration of antibiotics prior to surgery is associated with culture negativity (13, 14). Although eight of the culture-negative patients (67%) compared to 49 (49%) culture-positive PJI cases had received antibiotics in the 4 weeks prior, this difference was not statistically significant (Fisher's exact test, $P = 0.362$). Although multiplex PCR has been demonstrated to improve diagnostic sensitivity, it was not routinely employed in our study cohort (15, 16).

There are a number of limitations with the current study. First, the sensitivity and specificity were examined based on the cumulative number of specimens, such as when "up to five specimens" were obtained. This was necessary as there were insufficient data for reliable and meaningful analysis according to each stratum of culture numbers—for example, only three subjects had eight periprosthetic tissue samples obtained. We also observed that patients meeting the MSIS criteria for PJI had a higher number of specimens obtained than those not meeting these criteria for PJI; a similar observation was reported by Atkins et al. (5). A third limitation is the overall lower sensitivity, specificity, and accuracy when frequentist analysis was applied; this is an

artifact of the analysis as the "reference standard" specifically excluded microbiological criteria to avoid circularity and also reflects the stricter definition of a positive culture result. Likewise, latent class modeling is likely to yield optimistic results. Therefore, the interpretation of the results presented should be examined according to the magnitude of change with each stratum rather than the absolute values. Hip and knee prosthetic joints were overrepresented in this cohort, with only 17% of patients undergoing revision on an upper limb prosthetic joint. Finally, we recognize that the association between *Propionibacterium acnes* and shoulder arthroplasty infection compounds the challenge of PJI diagnosis (17).

In conclusion, building on our prior work demonstrating improved sensitivity for PJI diagnosis with the inoculation of periprosthetic tissue samples into blood culture bottles (8), this study suggests that fewer specimens are required than with the conventional techniques for an accurate diagnosis, with the greatest accuracy observed with three periprosthetic tissue samples cultured compared to the five to six recommended in current guidelines.

MATERIALS AND METHODS

Study population. The study was conducted at Mayo Clinic, Rochester, MN, between August 2013 and April 2014 (8). All consecutive patients undergoing revision arthroplasty surgery were included, including patients undergoing reimplantation as part of one- or two-stage exchange for management of PJI. Patients were excluded if no microbiological samples were obtained.

Ethics approval. The study was approved by the Mayo Clinic Institutional Review Board (IRB 13-005302).

Definitions. A modified version of the MSIS criteria for PJI was applied (4). PJI was diagnosed if one or more of the following were present: (i) documented sinus tract communicating with the affected joint, (ii) isolation of an indistinguishable microorganism from two or more periprosthetic specimens, or (iii) three or more of the following present—(a) elevated serum C-reactive protein (>100 mg/liter for acute PJI and >10 mg/liter for chronic PJI) or elevated erythrocyte sedimentation rate (>30 mm/h), (b) elevated synovial leukocyte count (>10,000 cells/μl for acute PJI and >3,000 for chronic PJI), (c) elevated synovial polymorphonuclear leukocyte percentage (>90% for acute PJI and >80% for chronic PJI), (d) evidence of acute inflammation on histologic examination, and (e) isolation of a microorganism from a single periprosthetic specimen (4). Acute PJI was defined as infection occurring <30 days from implantation or in the event of hematogenous seeding of the prosthesis, and chronic PJI was defined as infection occurring after 30 days, according to criteria proposed by Tsukayama and colleagues (18). For the purposes of this study, when comparing sensitivity and specificity, microbiological criteria were omitted to avoid circularity.

Microbiological methods. Fluids and tissues were collected as previously described (17, 19, 20). Tissues were homogenized using a Seward Stomacher 80 Biomaster (Seward Inc., Port St. Lucie, FL) operated on high in 5 ml brain heart infusion broth for 1 min. With conventional methods, the homogenized sample was inoculated onto sheep blood, chocolate, and CDC anaerobic blood agars and into prereduced thioglycolate broth (BD Diagnostic Systems, Sparks, MD), as previously described (8). Aerobic cultures were incubated for 5 days, and anaerobic cultures were incubated for 14 days. For culture in blood culture bottles, the homogenized sample was inoculated into Bactec Plus Aerobic/F and Bactec Lytic/10 Anaerobic/F blood culture bottles and placed on a Bactec 9240 instrument (BD Diagnostic Systems) as previously described (8). Inoculation of blood culture bottles was performed after inoculation of agar plates and thioglycolate broth; in the event that <1 ml of brain heart infusion broth remained, blood culture bottles were not inoculated. Blood culture bottles were initially incubated for 7 days (6, 19); however, on the basis of ongoing review of emerging literature over the study period, the incubation period was extended to 14 days (21). Bottles were subcultured only if the instrument flagged positive. Culture techniques for synovial and sonicate fluid samples were as previously described (17, 19).

Statistical analysis. Proportions were compared using the chi-square test or Fisher's exact test. Continuous variables were analyzed using a *t* test or Wilcoxon rank sum test, as appropriate. In the absence of a recognized, consensus reference standard, test sensitivity, specificity, and accuracy were estimated using a Bayesian latent class model, as described by Dendukuri et al., appropriate for estimating diagnostic test properties where no reference standard test exists (22–24). As a sensitivity analysis, a conventional, frequentist receiver operating characteristic (ROC) accuracy analysis was conducted. Accuracy was calculated as the area under the ROC curve. For all analyses, a P value of <0.05 was considered significant. Bayesian latent class analysis was conducted using BayesLatentClassModel (BLCM) software, version 1.11.2 (24). ROC analysis was conducted in Stata version 14 (StataCorp, College Station, TX). For both the Bayesian latent class and the frequentist analyses, a result was considered "positive" only if the same microorganism was isolated in two or more specimens (4).

ACKNOWLEDGMENTS

R.P. reports grants from BioFire, Check-Points, Curetis, 3M, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Allergan, and The Medicines Company. R.P.

is a consultant to Curetis, Roche, Qvella, and Diaxonhit. In addition, R.P. has a patent on a *Bordetella pertussis*/*B. parapertussis* PCR assay with royalties paid by TIB, a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic, and a patent on an antibiofilm substance issued. R.P. serves on an Actelion data monitoring board. R.P. receives travel reimbursement and an editor's stipend from ASM and IDSA and honoraria from the USMLE, Up-to-Date, and the Infectious Diseases Board Review Course.

Trisha N. Peel was supported by the Richard Memorial Kemp Fellowship, Royal Australasian College of Physicians, and the National Health and Medical Research Council Medical Early Career Fellowship (APP1069734). Allen C. Cheng was supported by a National Health and Medical Research Council Career Development Fellowship 2 (APP1068732). Robin Patel was supported by the National Institutes of Health (R01 AR56647 and R01 AI91594).

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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